Effect of Sodium Chloride, pH and Temperature on Growth of *Shigella flexneri*

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ABSTRACT

A systematic study of the effect of sodium chloride (0.5, 2.0, 3.5, 5.0%), pH (7.5, 6.5, 5.5), and temperature (37, 28, 19, 10°C) on growth of *Shigella flexneri* is reported for the first time, using a factorial design. Experiments were done using Brain-Heart Infusion media inoculated to contain 1 x 10^3 cfu/ml and incubated on rotary shakers (150 rpm). Growth curves were plotted from the experimental data by means of the Gompertz equation, and growth rates, lag times, generation times, and maximum populations were derived for all variable combinations. Results indicated that the three variables interact to affect the growth of *S. flexneri*, and combinations of low temperature, low pH, and high sodium chloride contents are strongly inhibitory.

Shigella has been recognized in recent years as an important causative agent of foodborne gastrointestinal disease (*2,7*). Considerable attention has been given to investigations of the taxonomy, epidemiology, and virulence of *Shigella* species (*7*). However, few systematic studies have been carried out on the effects of various environmental and cultural conditions on the viability and growth of *Shigella*. The most extensive report is that of Fehlhaber (*1*) who reported the growth range for 21 strains of *S. flexneri* to be 10°C to 44°C in a nutrient broth and the mean range of pH values for growth at 37°C to be 5.04 to 9.19. The maximum concentration of sodium chloride that permitted growth of the 21 strains in nutrient agar was 3.78% at 37°C. Experiments dealing with survival of *Shigella* species in sea water indicated that these organisms may be resistant to high concentrations of sodium chloride (*5*). Survival of *Shigella* in various foods has received some attention (*6,8*). Apparently these bacteria are capable of surviving for considerable lengths of time, even in acidic foods (*6*).

Since the infectious dose is reported (*4*) to be quite low (10-100 organisms), it is essential to determine the role that food ingredients, processing parameters, and environmental conditions or storage conditions may play in determining the fate of *Shigella* in food and its ability to cause infection.

The purpose of this investigation is to determine the effect of sodium chloride, pH and temperature on *Shigella flexneri* cultured in a microbiological medium in order to provide some needed basic information which, in conjunction with the development of a computerized program, would be useful in predicting the behavior of *Shigella* species in food.

EXPERIMENTAL

Microorganism

Shigella flexneri 5348 (obtained from Dr. David W. Niesel, University of Texas Medical Branch, Galveston, Texas). To prepare the inoculum, the organism was cultured for 24 h in Brain-Heart Infusion (Difco) at 37°C, and the culture was diluted with 0.1% peptone water.

Experimental design

A factorial design was employed (3 x 4 x 4 x 3) to assess the effects of pH (7.5, 6.5, 5.5), sodium chloride (0.5, 2.0, 3.5, 5.0%), and temperature (37, 28, 19, 10°C). All variable combinations were replicated three times.

Culture technique

Brain-Heart Infusion broth (Difco) was supplemented with 0, 15, 30, or 45 g/l sodium chloride to produce total concentrations of 0.5, 2.0, 3.5, or 5.0% sodium chloride, respectively. pH was adjusted to 7.5, 6.5, or 5.5 using 1N NaOH or 1N HCl. The medium was then dispensed in 100 ml portions into 500 ml Erlenmeyer flasks. The flasks were capped with foam plugs and sterilized by autoclaving for 15 min at 15 psi at 121°C. All flasks were inoculated with 1 ml of a diluted 24 h culture of *S. flexneri* to an initial level of approximately 1 x 10^3 cfu/ml. All flasks were then incubated on a rotary shaker (150 rpm) at the desired temperature.

At appropriate intervals 5 ml samples were withdrawn

from each flask by means of a pipet and the microbial population was determined by surface plating on Tryptic Soy Agar (Difco) using a Spiral Plater (Spiral System Instruments, Inc., Bethesda, MD). The plates (in duplicate) were incubated for 24 h at 37°C and counted.

Curve fitting and statistical analyses

Growth curves were generated from the experimental data using the Gompertz equation (*3*) (Table 1) in conjunction with ABACUS, a nonlinear regression program that employs a Gauss-Newton iteration procedure. This FORTRAN-based program was developed by W. C. Damert (U.S. Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA), and copies are available upon request. The Gompertz parameter values (A, B, C, M) were subsequently used to calculate lag times (h), growth rates [($\log_{10}$ cfu/ml)/h], generation times (h), and maximum population densities ($\log_{10}$ cfu/ml) as described by Gibson *et al. (3)*.

TABLE 1. *Gompertz equation.*

$$L(t) = A + C \exp [- \exp (- B (t - M))]$$

L(t) = the log count of the number of bacteria at time t (in hours).
A = the asymptotic log count as t decreases indefinitely.
C = the asymptotic amount of growth (log number) that occurs as t increases indefinitely.
M = the time (in hours) at which the absolute growth rate is maximum.
B = the relative growth rate at M.

Associated equations:

Exponential growth rate [($\log_{10}$ cfu/ml)/h] = BC/e.
Lag phase duration (h) = M - (1/B).
Generation time (h) = ($\log_{10}$2e)/BC.
Maximum population density ($\log_{10}$ cfu/ml) = A + C.

RESULTS AND DISCUSSION

Bacterial growth curves were calculated from the experimental data for each variable combination using the Gompertz equation (*3*) (Table 1). An example of the types of growth curves obtained is shown in Fig. 1, which illustrates the effect of increasing sodium chloride concentration on the rate and extent of growth of *Shigella flexneri*. The Gompertz equation parameters (A, B, C, M) obtained were subsequently used to calculate growth rates, generation times, lag times, and maximum population densities (Table 2).

The maximum growth rate was obtained at 37°C. Growth did not occur at 10°C; thus, observations obtained at 10°C are not included in Table 2. Growth rates in cultures at pH 5.5 were lower than those in cultures at pH 6.5 and 7.5. The organism failed to grow at 19°C and pH 5.5 even in media without added sodium chloride. Increasing levels of sodium chloride progressively decreased growth rates and increased generation times and lag times. However, when growth occurred, the maximum population densities were high, even in the presence of up to 3.5% sodium chloride. Optimum growth was obtained in cultures of pH 6.5

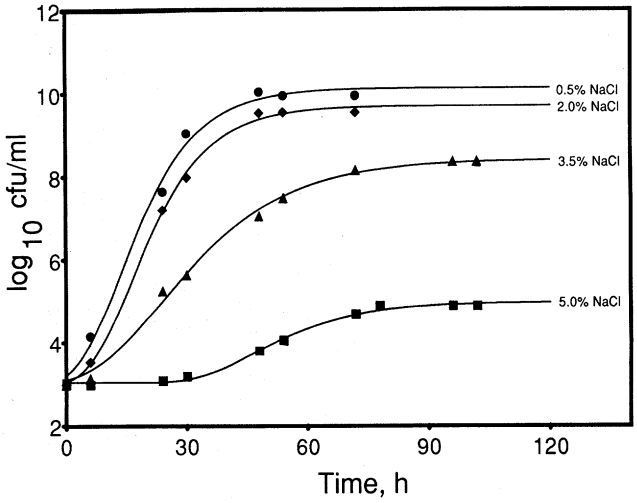

Figure 1. *Effect of NaCl concentration on grotwh of* S. flexneri *in BHI medium of pH 5.5 at 28°C.*

Growth rates of *S. flexneri* cultures under various conditions of temperature and sodium chloride content in media of pH 5.5, 6.5, and 7.5 are shown in Figs. 1, 2, and 3, respectively. Low temperatures and high sodium chloride concentrations interacted strongly to decrease growth rates at all pH values studied.

The values for growth rate, lag time and maximum populations density were used to calculate, by means of the Gompertz equation, the time needed to obtain a target bacterial population level (Table 3). This gave a measure of the combined effect of temperature, pH and sodium chloride concentration on the lag and exponential growth phases of *S. flexneri*. For example, *S. flexneri* present at a population level of 1 cfu/ml in a medium containing 2% NaCl, pH 6.5 at 28°C may be expected to reach a population level of 10,000 cfu/ml in approximately 11.5 h.

Predictions based on studies in microbiological media are useful in providing an indication of the effect that a given parameter, such as pH, salt content, or temperature, may have on the behavior of a microorganism in foods. However, other critical factors, such as the physical nature of the food, food components, additives or processing conditions, may cause deviations from these predictions. Thus, on the basis of information obtained in the present study, *Shigella* would be likely to grow in foods which are processed or stored at or slightly above room temperature and which contain normally encountered levels of sodium chloride (up to 3.5%). At slightly lower temperature (19°C) the bacteria would also be expected to grow in foods of neutral pH, while in acidic foods growth may be significantly inhibited.

Shigella would not be expected to proliferate at refrigeration temperatures (10°C and below) even in foods of low salt content, although there are indications (*6*) that the bacteria may remain viable for considerable periods of time. It should be noted that these predictions may strongly depend on the species of *Shigella* present.

TABLE 2. *Gompertz equation parameters and calculated growth curve values for* Shigella flexneri *cultured in BHI medium under various combinations of temperature, pH and sodium chloride concentration.*

Temp. (°C)	pH	NaCl (%)	A	C	B	M	Growth rate (log_{10} cfu/ml)/h	Generation time, h	Lag time, h	Max. pop. density log_{10} cfu/ml
37	5.5	0.5	2.31	7.47	0.198	6.40	0.54	0.6	1.3	9.8
		2.0	2.31	7.26	0.176	7.21	0.47	0.6	1.5	9.6
		3.5	2.31	6.54	0.161	9.86	0.39	0.8	3.7	8.8
		5.0	2.84	5.00	0.060	34.80	0.11	2.7	18.1	7.8
37	6.5	0.5	4.53	4.47	0.493	3.68	0.81	0.4	1.7	9.0
		2.0	4.53	4.91	0.335	5.14	0.60	0.5	2.2	9.4
		3.5	3.37	5.00	0.267	7.61	0.49	0.6	3.9	8.4
		5.0	3.25	5.11	0.113	14.44	0.21	1.4	5.6	8.4
37	7.5	0.5	4.26	5.41	0.435	4.54	0.86	0.3	2.2	9.7
		2.0	4.26	5.30	0.331	6.13	0.65	0.5	3.1	9.6
		3.5	3.23	4.76	0.299	10.02	0.52	0.6	6.7	8.0
		5.0	2.48	5.69	0.103	21.47	0.22	1.4	11.7	8.2
28	5.5	0.5	3.00	7.00	0.098	13.00	0.25	1.2	2.8	10.0
		2.0	3.00	6.70	0.094	16.11	0.23	1.3	5.5	9.7
		3.5	3.00	5.41	0.057	23.82	0.11	2.6	6.4	8.4
		5.0	2.99	2.00	0.063	45.60	0.05	6.5	29.8	5.0
28	6.5	0.5	2.96	6.75	0.181	8.54	0.45	0.7	3.0	9.7
		2.0	2.96	6.54	0.157	10.54	0.38	0.8	4.2	9.5
		3.5	2.96	6.16	0.092	17.84	0.21	1.4	7.0	9.1
		5.0	3.03	4.40	0.035	37.20	0.06	5.3	8.6	7.4
28	7.5	0.5	2.94	6.62	0.190	8.52	0.46	0.7	3.2	9.6
		2.0	2.76	6.83	0.157	11.79	0.40	0.8	5.4	9.6
		3.5	2.94	6.92	0.090	26.08	0.23	1.3	15.0	9.9
		5.0	3.03	NG	0.000	--	--	--	--	--
19	5.5	0.5	2.67	NG	0.000	--	--	--	--	--
		2.0	2.69	NG	0.000	--	--	--	--	--
		3.5	2.60	NG	0.000	--	--	--	--	--
		5.0	2.62	NG	0.000	--	--	--	--	--
19	6.5	0.5	2.78	7.19	0.033	42.80	0.09	3.4	12.7	10.0
		2.0	2.75	7.00	0.033	48.33	0.08	3.6	17.6	9.8
		3.5	2.80	6.20	0.009	160.10	0.02	14.2	52.6	9.0
		5.0	2.70	NG	0.000	--	--	--	--	--
19	7.5	0.5	2.90	7.16	0.035	43.40	0.09	3.3	14.7	10.1
		2.0	2.90	7.00	0.033	49.99	0.08	3.5	19.7	9.9
		3.5	2.41	5.24	0.049	257.73	0.10	3.2	237.4	7.7
		5.0	2.83	NG	0.000	--	--	--	--	--

NG = no growth.

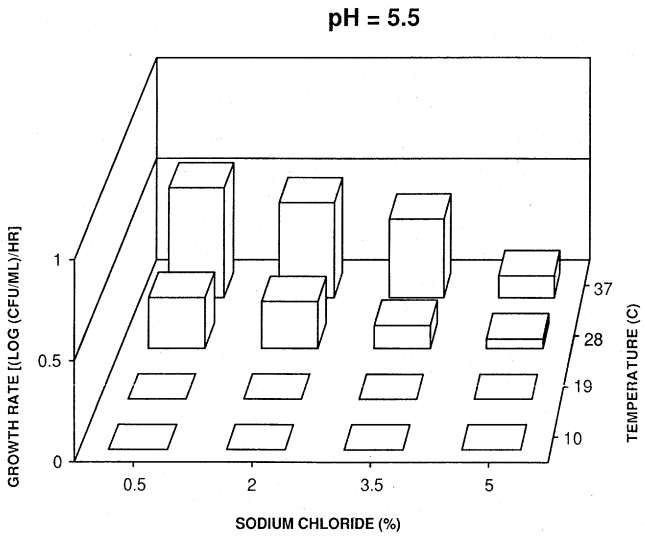

Figure 2. *Growth rates of* S. flexneri *in BHI media of pH 5.5 under various conditions of NaCl content (0.5, 2.0, 3.5, and 5.0%) and temperature 37, 28, 19, and 10 °C).*

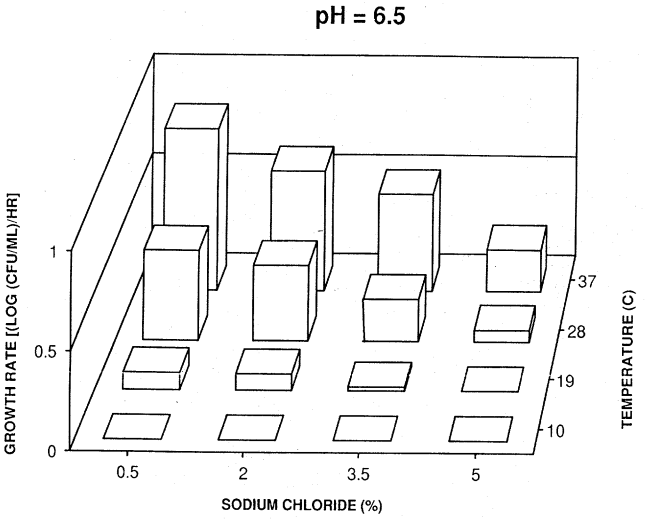

Figure 3. *Growth rates of* S. flexneri *in BHI media of pH 6.5 under various conditions of NaCl content (0.5, 2.0, 3.5, and 5.0%) and temperature (37, 28, 18, and 10 °C).*

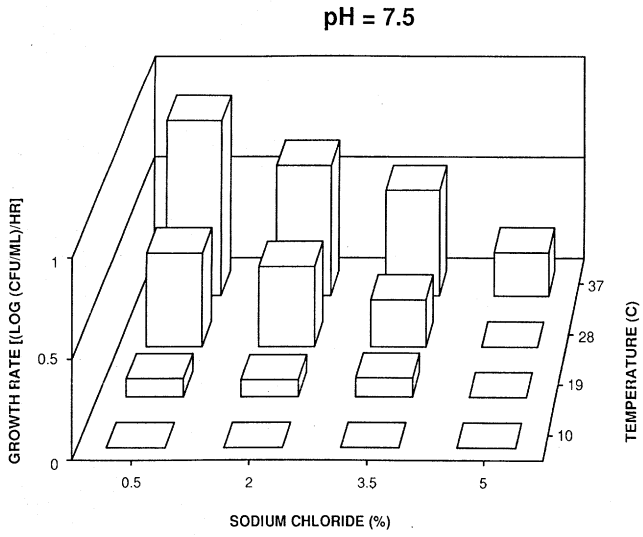

Figure 4. *Growth rates of* S. flexneri *in BHI media of pH 7.5 under various conditions of NaCl content (0.5, 2.0, 3.5, and 5.0%) and temperature 37, 28, 19, and 10 °C).*

TABLE 3. *Estimated time (h) to go from 1 cfu/ml to 10,000 cfu/ml.*

	NaCl (%)	pH 5.5	pH 6.5	pH 7.5
37°C				
	0.5	7.0	4.1	4.8
	2.0	8.0	5.6	6.5
	3.5	11.3	8.7	11.2
	5.0	41.4	17.1	24.7
28°C				
	0.5	13.9	9.2	9.2
	2.0	17.4	11.5	12.6
	3.5	29.0	19.9	27.2
	5.0	69.4	50.9	NG
19°C				
	0.5	NG	45.5	45.7
	2.0	NG	51.9	53.0
	3.5	NG	182.6	266.5
	5.0	NG	NG	NG

NG = no growth.

Analysis of the interactions of the parameters studied pon'des information on changes in growth rates as a result of alterations in pH, temperature, or sodium chloride concentration, and will be useful in determining conditions inhibitory to the growth and/or survival of *S. flexneri*. Data obtained, in conjunction with other experiments currently being carried out in our laboratory, will be used to develop computerized programs to model the growth of *Shigella* in foods.

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